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Deprotection and cleavage of peptides bound to Merrifield resin by stable dimethyl ether–poly(hydrogen fluoride) (DMEPHF) complex. a new and convenient reagent for peptide chemistry†

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The newly developed stable DMEPHF (1/15) complex was found to be a highly effective reagent for the cleavage of peptides from Merrifield resins; ease of handling and its simple, complete removal from the reaction mixture make the reagent system a very useful HF equivalent for applications in solid-phase peptide synthesis.

The fundamental coupling step in peptide synthesis was carried out more than 100 years ago, when Curtius[1] and later Fischer[2] synthesized the first simple peptide derivatives. Since the discovery of the importance of peptides in living systems the field has dramatically expanded. The isolation[3] and synthesis[4] of oxytocin and subsequent structure elucidation of insulin[5] promoted an unprecedented impetus for the development of new and effective peptide synthesis procedures.[6] The concept of Merrifield's solid-phase peptide synthesis[7] is the most successful of the techniques and recently has been almost exclusively used for the rapid synthesis of peptides. The synthesis of peptides requires a final cleavage of the peptide ester bond from the resin as well as the removal of the protecting groups.[8] Although, several methods have been developed for this purpose, the acidolysis is the most widely used method. The originally applied method[7] for breaking the ester bond between the peptide and the resin has been refined. Weaker acids have been replaced by the strong HF,[9,10] which cleaves not only the peptide–resin bond but simultaneously also removes the relatively acid resistant protecting groups. The most general reagent for this purpose is neat, liquid HF.[9] However, since HF is very corrosive, hazardous, and has a low boiling point (19.6 °C) effective alternatives were developed, such as pyridinium–poly(hydrogen fluoride) (PPHF, Olah's reagent).[11] PPHF was originally developed for fluorination reactions, however, later was applied to peptide chemistry as well,[12,13] mostly for removal of the protecting groups, including the acidolysis of the NO_2 group.[13] However, the presently used method of cleavage still requires the condensation of hydrogen fluoride and the use of different additives.[14,15] Recently, we described a new generation of stable HF–dialkyl ether reagents and successfully applied them in fluorinations.[16] The dimethyl ether–HF complex was found to be the most useful reagent based on its high stability, ease of handling and very convenient workup. Since both components are gases at ambient temperature, after the reaction the excess reagent can be completely removed from the reaction mixture leading to easy and convenient product isolation.

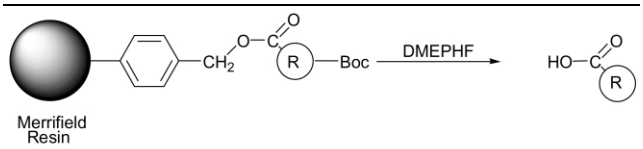
We now report application of the dimethyl ether–poly(hydrogen fluoride) reagent in peptide chemistry and describe its use in the convenient and effective deprotection and cleavage of peptides from resins. Since it was found that the DME to HF ratio strongly determined the reagent system's effectiveness we studied this aspect first.

DMEPHF complexes of different molar ratios were prepared and applied for the cleavage of lysine from Merrifield resin. It was observed that a minimum ratio of HF to DME of 5 was needed to carry out effective cleavage. Increasing the HF ratio resulted in more efficient cleavage, complexes with a ratio of 15 or 20 showed the best performance. The results are shown in Table 1.

It is important to note that these complexes are still sufficiently stable; they can be stored at room temperature for an extended period of time (months). Working with freshly prepared reagents, however, the cleavage took place with highest yields and selectivity. In agreement with the literature[14] results, simultaneous removal of protecting groups also occurred. However, similar to Merrifield's observations using thioethers as additives,[14] we also observed methyl ester formation using either lower HF ratios (Table 1) or longer stored (aged) reagents. The ^{1}H NMR studies have indicated[17] that the DMEPHF reagent stored at ambient temperature undergoes slow hydrolysis to form a CH_3OH-$[HF]_n$ complex. In the ^{1}H NMR spectrum a small but significant peak developed at 3.66 ppm (CH_3OH-$(HF)_5$) in addition to the original 3.51 ppm signal (CH_3OCH_3-$(HF)_5$). The ratio of these signals is about 1 to 100, however, with changes in the experimental conditions (storage time, DME–HF ratio *etc*.), the intensity of the CH_3OH-HF peak grew. It is worth noting that this complex was also found to be stable in our earlier study.[16] The formation of CH_3OH as an active methylating agent in the system explains the appearance of the methyl esters in the products. The ratio of methyl ester compared to free acid increased with storage time. Since the ester formation is an undesired side reaction, parallel experiments for the cleavage of Boc–Ile–Merrifield resin have been carried out with reagents kept at room temperature and at −20 °C. The comparative results are tabulated in Table 2.

As shown, the refrigerated reagent exclusively produced the expected free acid (Ile·HF) even after 25 weeks of storage, while the reagent kept at room temperature gave a significant amount of IleOMe·HF; its amount increased as the storage time extended. As a result, although the complex is stable and can be stored at room temperature, to ensure selective reaction and avoid methyl ester formation the reagent should be stored in a refrigerator. In light of these data the DMEPHF = 1/15 complex has been selected to illustrate the general applicability

Table 1 Effect of HF to DME ratio on the yield and selectivity of the cleavage of Boc–Lys(2-Cl-Z)–Merrifield resin



HF to DME ratio	Lys·HF (%)	LysOMe·HF (%)	Yield[a] (%)
1–3	—	—	0
4	75	25	5
5	92	8	92
10	98	2	91
15	100	0	92
20	100	0	93

[a] Isolated yields.

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Table 2 Effect of storage temperaure and time on the yield and selectivity of the cleavage of Boc–Ile–Merrifield Resin

Time of storage/week	Storage temperature/°C	Ile·HF (%)	IleOMe·HF (%)	Yield[a] (%)
0 (freshly prepared)	—	100	0	93
1	22	93	7	92
1	−20	100	0	92
2	22	88	12	91
2	−20	100	0	93
4	22	86	14	92
4	−20	100	0	93
10	22	82	18	91
10	−20	100	0	92
25	22	78	22	91
25	−20	100	0	93

[a] Isolated yields.

of the reagent. Different peptide–Merrifield resin complexes were cleaved according to a simple general procedure.‡ The protecting groups were as follows: Bzl-(Ser), Tos-(Arg), 4-MeBzl (Cys), 2-Br-Z-(Tyr), 2-Cl-Z (Lys), Fmoc- (Lys, entry 3). The results are tabulated in Table 3.

Table 3 Cleavage and deprotection of various amino acid– and peptide–Merrifield Resin (MR) complexes by DMEPHF (1/15) reagent

	Resin complex	Yield[a] (%)
1	Boc–Ile–MR	93
2	Boc–Ala–MR	94
3	Boc–Lys–MR	91
4	Boc–Cys–MR	91
5	Boc–Lys–MR	92
6	Boc–Gly–Pro–MR	93
7	Boc–Ala–Ile–MR	92
8	Boc–Gly–Ala–Ile–MR	93
9	NH_2–Ala–His–Glu–Trp–MR	88
10	Ac–Trp–Ser–Ser–Ser–MR	91
11	NH_2–Pro–Pro–Gly–Gly–Gly–MR	90
12	Ac–Tyr–Pro–Pro–Glu–Glu–MR	92
13	Ac–Ala–His–Arg–Thr–Cys–MR	95
14	NH_2–Gly–Ser–Met–Lys–Gly–Ala–Ile–Ile–Gly–Leu–Met–MR	89

[a] Isolated yields.

As NMR and MS studies showed, DMEPHF removed the bonded units from the resin, and the corresponding products could be isolated in excellent yields. During cleavage the protecting groups were also removed. In agreement with the literature[15] the exceptions are Ac and Fmoc groups, which remained attached (Table 3, entries 3, 10, 12, 13).

In conclusion, the newly developed DMEPHF (1/15) complex was found to be a convenient, stable and highly effective reagent for cleavage of peptides from Merrifield resin. Its ease of handling, simple and complete removal from the reaction mixture make it the reagent of choice as an HF substitute for applications in peptide synthesis.

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Notes and references

† Considered Synthetic Methods and Reactions, Part 206. For Part 205, see ref. 16.

‡ *General procedure for the cleavage of peptides from Merrifield resin using the DMEPHF (1/15) reagent system*: 0.5 g of resin-complex were suspended in 6 mL of DMEPHF (1/15) at −78 °C in a 30 mL Nalgene bottle. The mixture was stirred at 0 °C for 1 h, then the reagent was removed by passing a slow stream of dry nitrogen gas through the system. The remaining solid was stirred with 10 mL of deionized water for 10 min and the resin was removed by filtration. After the evaporation of the solvent the product was isolated as white powder/crystals. The products were dissolved in D_2O and analyzed by ^1H and ^{13}C NMR spectroscopy.

Tetra and higher peptide–resin complexes were cleaved by using anisole, cresole, thiocresole and Me_2S as scavengers. In some cases the cleavage was carried out with and without using scavengers for comparison. In the case of Table 3, entry 9 the scavengers had no effect, the product was free of any byproducts, using DMEPHF itself. However, in the case of Table 3, entry 13, the use of scavengers improved the product purity and their use is recommended when protected amino acids are in the peptide chain, similarly to the regular HF cleavage process. After cleavage the reagent was removed as above and the mixture was washed with ether and the peptide was dissolved in 10% aqueous AcOH and lyophylized.

In the case of higher peptides a Finnigan TSQ70000 mass spectrometer equipped with electrospray ionization (ESI) source was used for analysis.

Caution: anhydrous HF is an extremely corrosive and low boiling gas (19.5 °C) and should be handled in a well ventilated hood with protective gloves, face mask and clothing. Using DME to stabilize HF will produce a thermally stable complex, however, the use of the complex still requires extreme caution, and should be considered as HF with higher boiling point. For more details on handling see ref. 16.

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